DON (VOMITOXIN) TESTING SERVICES

1. PURPOSE

This directive establishes official procedures for determining Deoxynivalenol (DON) in grain and certifying the official results. This service is provided as official criteria under the authority of the United States Grain Standards Act (USGSA), as amended.

2. REPLACEMENT HIGHLIGHTS

This directive is issued to: revise the instructions for the Romer - DON Fluoroquant™ method of testing; provide testing procedures for the Romer - AccuTox™ test method; change all references from vomitoxin to DON; and to provide safety requirements for table-top and laboratory testing. Additionally, the certification section is revised to clarify reporting and certification options. This directive supersedes FGIS Program Directive 9180.57, dated 10/27/97.

3. GENERAL INFORMATION

DON, also referred to as vomitoxin, is a naturally occurring mycotoxin produced by several species of Fusarium. Wet and cool weather from flowering time on to maturity promotes infection, resulting in scab or head blight in barley, wheat, oats, and rye.

The Federal Grain Inspection Service (FGIS) of the Grain Inspection, Packers and Stockyards Administration (GIPSA) provides DON testing service as official criteria for wheat, barley, oats, and corn. DON testing is available as a qualitative and as a quantitative service using test kits supplied by Neogen and Romer.[1/] All official DON testing of grain is performed as prescribed in this directive by authorized employees of FGIS or licensed delegated/designated agency personnel.

Individuals wanting grains officially tested for DON should contact the nearest FGIS field office or delegated/designated agency.

1/ The mention of firm names or trade products does not imply that the U. S. Department of Agriculture endorses or recommends them over other firms or similar products not mentioned.

DON test results are not reported to the Food and Drug Administration (FDA) because action limits are not established at this time.

The methods listed below have been conformance tested to perform within FGIS specifications. Each of the approved test methods has been certified to provide results accurate up to the conformance test level at which they were approved. Any test results that are above the established conformance limits are reported as estimates unless a supplemental analysis is performed.

FGIS APPROVED TEST METHODS			
Method and Test Kit	Approved for		Conformance Limit
	Qualitative	Quantitative	
AgriScreen (Neogen)	X		5 PPM
Veratox (Neogen)	X	X	5 PPM
Fluoroquant (Romer)	X	X	5 PPM
AccuTox™ (Romer)	X	X	5 PPM

The following chart lists the DON field test kits and the grains/commodities for which they have been approved. For information concerning the testing of other grains/commodities, contact the Standards and Procedures Branch.

GRAIN/ COMMODITY	TEST METHOD			
	DON Fluoroquant	Veratox	DON AccuTox	AgriScreen
Barley	X	X	X	X
Malted Barley	X	X	X	X
Corn	X	X	X	X
Oats	X	X	X	X
Wheat	X	X	X	X
Wheat Flour			X	
Wheat Midds			X	

4. WORK AREA REQUIREMENTS

The work area requirements covered under this section apply to FGIS-occupied space only.

a. Sample Grinding Area. Samples must be ground in space separate from the analytical space. The field office manager and safety officer must determine whether added ventilation or a dust removal device is needed in the grinding area to remove airborne dust particles. Refer to the FGIS Safety and Health Office in Washington, D.C. for assistance in determining whether added dust removal equipment (e.g., exhaust fan) is required.

b. Sample Testing Area. Test methods that involve the use of volatile chemicals (e.g., acetonitrile, methanol) must be performed in FGIS-approved laboratory space. Testing methods that are free from hazardous materials may be performed (upon approval of the field office manager) in alternate locations. The field office manager and safety officer must evaluate the testing materials to determine if FGIS-approved laboratory space is required. If testing is performed in an alternate location (i.e., table-top in inspection lab), consideration must be given to lighting, plumbing, electrical, and ventilation requirements. Refer to the FGIS Safety and Health Office in Washington, D.C. for assistance in determining if FGIS-approved laboratory space is required and whether added ventilation (e.g., exhaust fan) is required for alternate testing locations.

5. SAFETY

FGIS employees must comply with good practices to ensure a safe and efficient work environment. To accomplish this, include the following as part of an overall FGIS laboratory/testing area "Standard Operating Procedure" (SOP). Maintain the SOP, this directive, and current Material Safety Data Sheets (MSDS) at each laboratory/testing location.

During onsite supervision at agency locations, FGIS employees must assess their personal safety requirements. If personal safety is questionable, FGIS employees must determine if personal protective equipment can be used to correct the safety deficiency at the testing location. If FGIS employees cannot utilize personal protective equipment to provide for a safe work environment, then onsite DON supervision must occur only when the testing area is considered safe.

Interested persons are restricted from entering the DON testing area during testing unless accompanied by official personnel and must observe the health and safety rules while in the area.

a. General Safety Practices.

(1) Table-Top Testing. FGIS personnel must abide by the following safety practices when performing testing in an alternate location (e.g., table-top in inspection lab).

(a) Do not smoke, eat, drink, or chew gum or tobacco in the immediate testing area.

(b) Wash hands immediately before and after eating, drinking, and smoking.

(c) Wear the following protective equipment when testing is being performed: disposable, fire-retardant laboratory coat; disposable, impermeable gloves; safety glasses or splash goggles.

(d) Wear a disposable mask (3M model 8511) or Moldex Series 2300N (Models 2300N95, 2301N95, 2307N95) and hair protection (Lab Safety Supply EB 1357 or 1356) when exposed to airborne grain dust.

(e) Do not store food or drink in the refrigerator used for storing chemicals and solutions, and test kits.

(f) Do not store masks and hair protectors in the grinding area where they might become contaminated by the dust particles.

(2) Laboratory Testing. FGIS personnel must abide by the following safety practices when performing testing in an FGIS-approved laboratory.

(a) Do not smoke, eat, drink, or chew gum or tobacco in the laboratory.

(b) Wash hands immediately before and after eating, drinking, and smoking.

(c) Wear the following protective equipment: disposable, fire-retardant laboratory coat; disposable, impermeable gloves; safety glasses or splash goggles.

(d) Wear a disposable mask (3M model 8511) or Moldex Series 2300N (Models 2300N95, 2301N95, 2307N95) and hair protection (Lab Safety Supply EB 1357 or 1356) when exposed to airborne grain dust.

(e) Do not wear contact lenses in the immediate testing area.

(f) Do not store food or drink in the laboratory refrigerator used for storing chemicals, solutions, and test kits.

(g) Do not store masks and hair protectors in the grinding area where they might become contaminated by the dust particles.

(h) Label all bottles and containers according to the Hazard Communication Program and the Chemical Hygiene Plan. In addition, when preparing mixtures of solutions, securely apply a label with the name of the solution, the preparation date, and the preparer's initials written in permanent ink.

(i) Store equipment outside the fume hood in a manner that will not clutter bench tops or obstruct movement.

(j) Prepare all chemical solutions and perform chemical analyses under a working fume hood.

(k) Limit the total quantity of waste chemicals in the laboratory to one liquid gallon.

(l) Limit the total amount of flammable solvent (including waste) in the laboratory to two gallons.

(m) Maintain a current MSDS for each chemical in the laboratory. If each supply of chemicals received does not have an MSDS enclosed, contact the company and request one immediately.

(n) Store flammable solvents in an approved storage cabinet.

(o) Store waste chemicals (e.g., acetonitrile, methanol) in impermeable metal containers meeting Underwriters Laboratory approval for Class I liquids. The containers must be capable of maintaining a tight seal and must be labeled "Flammable" or "Biohazardous Material" or both, as applicable.

(p) Contact an Environmental Protection Agency (EPA)-approved or EPA-certified waste disposal company and make arrangements for removal of chemical wastes or provide other suitable waste disposal procedures consistent with existing laws that do not create a hazard to the community.

6. SANITATION REQUIREMENTS

The sanitation requirements for spillage, labware, and excess sample extract listed in this section are applicable to testing performed at an FGIS-approved laboratory or an alternate testing location (e.g., table-top in the inspection lab).

Official agencies must adhere to the requirements for spillage and labware and should follow procedures established in their area for the disposal of excess sample extract.

Perform the following procedures only while wearing disposable, impermeable gloves, chemical splash goggles, and a fire-retardant laboratory coat. If hands become contaminated, wash immediately with soap and water.

a. Spillage. Clean areas and materials contaminated by any DON solution spills. Wipe up the affected areas using an absorbent cloth or paper towels, then wash the area with a soap/water solution. Place cleaning materials in a plastic waste bag, close tightly, and discard in a dumpster or landfill disposal site.

b. Labware. Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

c. Excess Sample Extract. The disposition of excess sample extracts and solutions varies with the testing methodologies. All sample extracts containing chemicals such as methanol and acetonitrile are treated as hazardous chemicals and are disposed of in the chemical waste container. Unused extracts consisting of water only or a water/salt solution may be disposed of by pouring down the drain. Refer to the appropriate testing procedures for specific waste disposal instructions.

7. FGIS LABORATORY REQUIREMENTS

FGIS-approved laboratories are required for mycotoxin testing that involves the use of hazardous materials (e.g., flammable liquids). The requirements covered under this section apply to FGIS-occupied space that is dedicated for the sole function of mycotoxin testing.

Some DON testing methods require the use of flammable liquids and suspected carcinogens. The building owner (private or GSA) must permit the use of chemicals (e.g., acetonitrile, methanol) in space used by FGIS. FGIS will provide testing services onsite only in facilities that provide protection to FGIS personnel.

Individual elevators may provide two kinds of space for FGIS personnel to perform onsite DON testing. The space may be located (1) in a building along with other occupants, or (2) in a building devoted exclusively to laboratory space.

In either case, the plan for the intended laboratory space is subject to inspection and approval by FGIS prior to construction. The Safety and Health Office and field office manager will review proposed plans and suggest ways to comply with the requirements.

The following are minimum requirements for FGIS-occupied laboratory space.

a. Location. Locate the laboratory at least 100 feet from the base of the elevator headhouse. This distance is subject to negotiation when the elevator uses exterior grain legs and/or inclined belts in lieu of interior grain legs or where the headhouse is equipped with blow-out panels or the headhouse consists of a lightly covered framework.

Laboratories must meet the following requirements when they are located in a building with other occupants:

(1) Isolate the laboratory from nonlaboratory occupants using a fire barrier having at least a 1-hour fire resistance.

(2) Provide a fire barrier consisting of floors, ceilings, and interior walls.

(3) Provide all passageways and other openings that lead to adjacent interior space with self-closing fire doors having a 1-hour fire resistance. Do not block these doors open.

(4) Separate the space from central heating, ventilation, and air-conditioning using automatic-closing fire dampers in the heating, ventilation, and air-conditioning ducts near the fire-barrier, or provide a separate heating, ventilation, and air-conditioning system in the laboratory.

b. Size. Dedicate the space strictly for laboratory (chemical) work. Supply adequate space for chemical analysis (minimum of 100 square feet).

c. Electrical System. Provide the laboratory space with electrical power and lighting meeting the standards of the National Electrical Code. Wiring suitable for Class I location is not required. A three-wire system consisting of an energized wire, a neutral wire, and a grounding conductor is satisfactory. Install overhead lighting fixtures through ceilings that serve as fire barriers. Fixtures suspended below such ceilings are acceptable.

d. Plumbing. Provide the laboratory space with a basin having hot and cold potable water and a sewer connection.

e. Exhaust System. The exhaust system must remove chemical vapors from the work area. Normal air conditioning and heating may provide adequate ventilation when performing testing procedures in a building devoted exclusively for laboratory space. Offices that use the Romer Fluoroquant method must have an explosion proof exhaust system in place. Refer to the FGIS Safety and Health Office in Washington, D.C. for assistance in determining whether added ventilation, such as a fume hood, is needed. If needed, situate the laboratory space so that hoods are vented to the exterior of the building. Fume hood ventilation will require a 6- or 8-inch diameter opening, either vertically through the ceiling and roof or horizontally through an exterior wall. In some cases, a portable hood may be sufficient.

f. Eyewash and Safety Shower Station. Provide the laboratory space with eyewash equipment (eyewash bottle or permanent faucet-mounted fixture). A permanent, faucet-mounted eyewash fixture is highly recommended. A safety shower station must be installed in laboratories where acetonitrile-based extraction solvent (Romer-Fluoroquant test method) is used.

g. Cautionary Markings. Provide signs for the laboratory door(s) as follows:

(1) "Biohazardous Material Present"

(2) "No Smoking, Eating, or Drinking"

(3) "Flammable Material Present"

(4) "Wear Safety Protection"

(5) "Admittance of Authorized Personnel Only"

(6) Refrigerator Signs. Provide signs for the refrigerator used for storing test kits, chemicals, or solutions, as follows:

(a) "Biohazardous Material Present"

(b) "No Food or Drink to be Stored in this Refrigerator"

For further information concerning the laboratory space requirements, contact the FGIS Safety and Health Office.

8. TYPES OF SERVICES

Applicants requesting DON testing must specify whether qualitative or quantitative testing service is desired. If qualitative analysis is requested, the applicant must specify the level desired (e.g., 1, 2 ppm). Three types of DON testing services are available as follows:

a. Submitted Sample Service. Analysis based on a sample submitted by the applicant for service.

b. Official Sample-Lot Service. Analysis based on an official sample obtained and analyzed by official personnel.

Unit trains and shiplots are analyzed on a sublot basis for wheat and barley and on a composite basis for other grains. (Unit train analysis shall be limited to a maximum of five carriers per sublot.) Acceptable sublots must conform to contract specifications when "maximum" limits are specified.

(1) Supplemental Testing.

Upon request, supplemental testing may be performed as follows:

Composite samples may be analyzed in addition to the sublot test for wheat and barley shiplots or unit trains.

(2) Alternate Testing.

Upon request, alternate testing methods may be used, provided that the minimum testing requirements are met. Examples of alternate testing are as follows:

(a) Sublot testing may be used instead of composite sample analysis for grains routinely tested on a composite basis.

(b) Grain shipments may be tested on a component sample basis in lieu of the sublot basis under the provisions of Book III, Inspection Procedures. Components are combined and averaged to determine the sublot result.

Component samples will not be designated as a material portion due to DON because the FDA has not established a reportable action level. Acceptable quality will be based on the sublot result as compared to the contracted "maximum" specification.

c. Warehouse Sample-Lot Inspection Service. Analysis based on an official sample obtained by a licensed warehouse sampler and analyzed by official personnel.

9. REVIEW INSPECTIONS

Sections 800.125 and 800.135 of the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factor and official criteria may be handled separately even though both sets of results are reported on the same certificate.

Review inspection services for DON are provided on either a new sample or the file sample in accordance with the regulations. Board appeal inspection services are limited to the analysis of file samples.

Only one field review (reinspection or appeal inspection) is permitted for shiplot, unit train, or lash barge material portions when testing is performed on a sublot basis. However, if the applicant requests a review of the entire lot, up to three review levels of service (reinspection, appeal, board appeal) may be obtained for each sublot included in the lot. Inspection results for each review level shall replace the previous inspection result.

a. Reinspection Service. The laboratory providing original testing services also provides reinspection services. Applicants may request either qualitative or quantitative analysis unless the original test was quantitative. Then, only a quantitative analysis is available.

b. Appeal Inspection Service. FGIS field offices provide appeal DON testing services. Field offices not equipped to provide testing will make arrangements with another FGIS office to provide the most timely service possible. Applicants may request either qualitative or quantitative analysis unless the original or reinspection tests were quantitative. Then, only a quantitative analysis is available.

If samples are sent to a field office for analysis, write the words **"DON APPEAL"** in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

c. Board Appeal Inspection Services. Board appeal inspection services are limited to the file sample and are provided by the Board of Appeals and Review (BAR) in Kansas City. Applicants may request either qualitative or quantitative analysis unless the original or reinspection tests were quantitative. Then, only a quantitative analysis is available. The High Pressure Liquid Chromatography (HPLC) method is also available for determining DON in Board appeal samples. The applicant must specify the HPLC method as the desired determination method. Otherwise, the Board appeal inspection will be conducted using the rapid method (test kits).

When sending samples to the BAR, write the words **"DON BOARD APPEAL"** in the "Remarks" section of the grain sample ticket and on the back of the mailing tag.

10. SAMPLE SIZE, PREPARATION, AND GRINDING

a. Sample Size and Preparation. A sample of approximately 200 grams, with dockage and stones removed, is required for the DON testing and file sample (100 grams work portion, 100 grams file portion). An additional sample may be required if subsequent review inspections are requested. A similar sample size is recommended for submitted samples.

Obtain samples according to the guidelines in the Grain Inspection Handbook, Book I, "Grain Sampling." Use a Boerner divider to obtain an approximately 100-gram portion for DON testing. Save the remaining sample portion as the file sample.

b. Grinding.

SAFETY NOTE: OPERATOR MUST OBSERVE SAFETY PRECAUTIONS AND WEAR EYE PROTECTION WHEN OPERATING THE GRINDER. SEE THE GRINDER'S MANUAL FOR MORE SAFETY TIPS.

Grind approximately 100 grams (dockage and stone free) of grain using a Romer Mill-Model 2a, Udy Grinder, Perten Falling Number Mill, Bunn Commercial Coffee Grinder, or an equivalent device that meets FGIS' performance requirements.

The grinding apparatus must be adjusted to produce a particle size that is sufficiently fine enough to obtain a homogeneous blend. Generally, a sufficiently coarsely ground sample of wheat resembles whole wheat flour, while a sample that is too coarsely ground has the appearance of bulgur or semolina. Avoid over-grinding or pulverizing a sample because it produces an excessively powdery mix that will slow down the filtration process.

To check the performance of equipment used for grinding **small grains (e.g., wheat and barley),** use the following procedures:

(1) Grind a sample portion of approximately 100 grams of relatively dry wheat (i.e., 13 percent or less moisture).

(2) Weigh the entire portion that was ground.

(3) Sieve the portion across a standard No. 20 wire woven sieve.

(4) Weigh the portion that passed through the sieve.

(5) Determine the percent of fine material, by weight, as follows:

Fines = weight from step (4) divided by the weight from step (2) X 100.

For locations that perform mycotoxin testing on coarse (e.g., corn) and small grains, perform the check using a 100-gram sample portion of corn having a moisture content of 14 percent or less.

The optimum range for particles of coarse and small grain passing through the No. 20 sieve is between 60 and 75 percent. Whenever the ground particles appear to be too coarse, or the results of a grinder check indicate that less than 50 percent of the ground portion passes through the No. 20 sieve, the grinder should be adjusted or repaired to meet the optimum range requirements.

Grinding apparatuses must be checked periodically to determine whether they are producing a final product that meets the particle size requirements as listed above. Official personnel shall determine the frequency of the checks based on a number of items that include visual observation of the ground product, number of samples ground since last check, and time (number of days) since the last check was performed. Record all particle check results in a convenient location for future reference purposes.

11. NEOGEN TEST KIT METHODS

The extraction solution and other materials used in the AgriScreen and Veratox test kits does not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this directive to ensure a safe and efficient work environment.

a. Extraction Procedures.

Procedures for Barley, Corn, Oats, and Wheat:

(1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker. A filtering syringe packed with glass fiber is also permitted in lieu of filter paper.

(2) Label the collection container with the sample identification.

(3) Thoroughly mix the ground sample and weigh a 50-gram portion.

(4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.

(5) Add 250 mL of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.

(6) Let material stand for 2 minutes to enable some of the sample to settle before filtering the extract.

(7) Filter the extract by pouring at least 15 mL through the filter paper.

Procedures for Malted Barley:

Follow step numbers 1-7 listed above, then pass 3 mL of the filtered extract through a Bond Elut SPE cartridge at a flow rate of 1 mL per minute.

b. Analysis Procedures.

Use the Neogen AgriScreen or Veratox test kits when performing the following:

(1) Preparation of Solutions.

(a) Open one of the conjugate bottles and remove rubber stopper. Cut the tip off the enclosed squeeze tube. Squeeze tube contents into the bottle. Replace the stopper and swirl contents until the pellet has dissolved. ALLOW THE REHYDRATED CONJUGATE SOLUTION TO SET FOR 1 HOUR PRIOR TO USE. Use the contents of the bottle until empty (**once rehydrated, contents must be used within 3 weeks**). Mix the second bottle of conjugate in the same manner when needed. KEEP REFRIGERATED WHEN NOT IN USE.

(b) Substrate is pre-activated, ready for use, and should be stored in the dark. Remove only one vial of substrate at a time from the foil pouch prior to use.

(c) Open the stopping reagents and the DON control bottles and set aside. Swirl to mix prior to use.

(2) Conducting the Test.

(a) Qualitative (Screening) using Neogen AgriScreen or Veratox Test Kits.

NOTE: The AgriScreen kit is supplied with a 1 ppm control. Users must purchase another control to perform screening at a different level.

1) Test Procedure.

a) Remove red-marked mixing well strip and break off needed number of wells (one well for each sample and one well for control). Return unused strip to package.

NOTE: Do not run more than six wells (five samples plus one control) at a time unless using a multichannel pipettor.

b) Remove antibody-coated well strip and break off same number of wells. Return unused strip to package and tightly close the package opening.

Mark one end of antibody-coated well strip with C for control so that you can identify wells after washing.

c) Firmly place a pipette tip on syringe and add 100 microliters (μl) of conjugate to each mixing well. Discard tip.

NOTE: 100 μl of liquid is the amount drawn into the pipette tip when the syringe plunger is depressed and then released slowly.

d) Remove the stopper from the control bottle. Firmly place a new pipette tip on syringe and add 100μl of the control to the first mixing well. Thoroughly mix by depressing plunger five times. Discard tip. Replace rubber stopper on control bottle.

e) Firmly place a new pipette top on syringe and add 100 μl from the sample collection tube to second well of red-marked mixing strip. Thoroughly mix by depressing plunger five times. Discard tip.

f) Repeat step e) for each additional sample.

g) Transfer 100 μl from each red-marked mixing well to corresponding antibody-coated well. Use a new tip for each well. Discard red-marked wells.

h) Mix by sliding wells back and forth on a flat surface in a manner to ensure adequate mixing (10 to 20 seconds) without splashing reagents from wells. **Wait 10 minutes** (begin time after mixing).

i) Initial reaction is now completed. Shake out the contents of antibody-coated wells.

j) Using a wash bottle, fill each antibody-coated well with distilled/deionized water and shake out. Repeat five times. Remove all water droplets by turning wells upside down and vigorously tapping wells on paper towel.

k) Firmly place a new pipette tip on syringe and add 100 μl of substrate to each antibody-coated well. Discard tip.

l) Mix as instructed in step h) and **wait 10 minutes** (begin time after mixing).

m) Firmly place a new pipette tip on syringe and add 100 μl of stop solution to each well. Discard tip. Mix by tapping gently on the side of the antibody well strip.

n) Visually determine the levels at 1 ppm or 2 ppm only or read the results in the Microwell Model EL 301 Strip Reader as follows:

2) Reading the Results.

a) Make sure that the Microwell reader is on and allowed to warm up for a minimum of 15 minutes before using.

b) Remove sample carriage and hit ENTER.

c) Insert W2 filter (405 rm) and hit ENTER.

d) Insert W1 filter (650 rm) and hit ENTER.

e) Hit CLEAR and then BLANK. This will cause the instrument to read air as the blank sample.

f) Load antibody-coated wells into sample carriage so that the control is in position A1.

g) Load the sample carriage into the strip reader so that position A1 is under the reader.

h) Hit READ and record the value obtained for A1 (the control).

i) Slide the carriage to position A2 and hit READ.

j) If the value is **EQUAL TO** or **LARGER THAN** that recorded for A1, the sample is **LESS THAN** or **EQUAL TO** the control. If the value is **SMALLER THAN** that recorded for A1, the sample contains **MORE THAN** the control.

k) Slide the carriage to the next sample and hit READ. Repeat step j) for each of the remaining samples.

3) Reporting and Certifying the Results.

a) Report results on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 2 ppm) or as exceeding the threshold.

b) Certify results as being equal to or less than a threshold. (See the Certification Section for detailed procedures and statements).

(b) Quantitative Determination.

Use the Neogen Veratox test kits when performing the following:

1) Test Procedures.

a) Remove red-marked mixing well strip and break off the number of wells needed (five wells for controls and one for each sample) up to a maximum of twelve. Mark one end of red-marked mixing well strip with a 0 (zero) for the blank and the other end with an S for samples so that you can identify the wells. Place wells in the well holder.

b) Remove an equal number of antibody-coated wells. Mark one end of strip with a 0 (zero) for the blank and the other end with an S for samples and place strip in the well holder with the 0 (zero) marked end on the left.

c) Mix each reagent by swirling the reagent bottle prior to use.

d) Firmly place a pipette tip on the 100 μl pipettor and add 100 μl of conjugate to each mixing well. Discard the tip.

e) Remove the cap from the 0 ppm control bottle. Firmly place a new pipette tip on the 100 μl pipettor and add 100 μl from the 0 ppm control bottle to the first (labeled 0 (zero)) mixing well. Discard tip and replace cap on control bottle.

f) Remove the cap from the 0.5 ppm control bottle. Firmly place a new pipette tip on the 100 μl pipettor and add 100 μl from the 0.5 ppm control bottle to the second mixing well. Discard tip and replace cap on control bottle.

g) Repeat step f) with the remaining control standards placing 100 μl amounts of these standards in the third, fourth, and fifth wells, respectively. A new pipette tip should be used for each standard solution.

h) Firmly place a new pipette tip on the 100 μl pipette and add 100 μl from the sample collection tube of the first sample to the sixth well. Discard tip.

i) Repeat step h) for each sample, placing 100 μl of extract from each sample in a different well. Use a new pipette tip for each sample solution.

j) Using a 12-channel pipettor with new tips, mix the wells by pipetting the liquid up and down in the tips three times. Transfer 100 μl to the antibody wells.

k) Mix by sliding the Microwell holder back and forth on flat surface in a manner to ensure mixing (10-20 seconds) without splashing reagents from wells. **Wait 10 minutes** (begin time after mixing). Discard red-marked wells.

l) Initial reaction is now completed. Shake out the contents of antibody-coated wells.

m) Using a wash bottle, fill each antibody-coated well with distilled water and shake out. Repeat five times. Remove all water droplets by turning wells upside down and vigorously tapping wells on paper towel.

n) Pipette 3 mL of substrate into the reagent boat and, with new tips on the 12-channel pipettor, pipette 100 μl of substrate into the wells and mix as instructed in step k). **Wait 10 minutes** (begin time after mixing).

o) Discard remaining substrate and rinse the reagent boat with water.

p) Pipette 3 mL of stop solution into the reagent boat. Using the same pipette tips as were used to dispense substrate, add 100 μl red stop to each well and mix thoroughly as instructed in step k). Discard tips.

2) Reading the Results.

NOTE: Connect the Microwell reader to a computer system. For FGIS computers, the computer must have the necessary software installed on the C drive, subdirectory "DON." Perform the following (computer procedures may vary for official agencies depending on how the software is installed).

a) Allow the Microwell to warm up for a minimum of 15 minutes before using.

b) Turn on the computer.

c) At C:\> prompt, type in CD\DON and press the ENTER key.

d) At C:\DON> prompt, type LL and press the ENTER key.

This will bring the MAIN MENU of the Log/Logit program on the computer screen.

e) Type A to select the "Run Log/Logit Program" option.

f) At, “Please Enter the Number of Standards:” type 5 and press the ENTER key.

g) At, “Enter Standard Units:” type ppm and press the ENTER key.

h) At, “Standard 2 Concentration:” type the concentration level (e.g., 0.5) and press the ENTER key.

i) At, “Standard 3 Concentration:” type concentration level (e.g., 1) and press the ENTER key.

j) At. “Standard 4 Concentration:” type concentration level (e.g., 2) and press ENTER key.

k) At, “Standard 5 Concentration:” type concentration level (e.g., 6) and press the ENTER key.

l) If all values are correctly entered, press the F1 key. If they are not, press the E key and follow the instructions on the screen to edit values. When all values are correct, hit the F1 key. **STOP! Do not use the computer keyboard until the samples have been read in step u).**

m) On the Microwell reader, remove the sample carriage and press the ENTER key on the Microwell reader.

n) Insert the W2 filter (405 nm) and press the ENTER key.

o) Insert W1 filter (650 nm) and press the ENTER key.

p) Press CLEAR and then BLANK. This will cause the instrument to read air as the blank sample.

q) Load antibody-coated wells into sample carriage so that the control labeled 0 (zero) is in position A1.

r) Load the sample carriage into the strip reader so that position A1 is under the reader.

s) Press READ and an absorbance value for A1 should appear in the screen on the Microwell reader.

t) Slide the carriage to position A2 and press READ. An absorbance value for A2 will appear.

u) Repeat step t) until absorbance values have been obtained for all controls and samples.

v) Return to the COMPUTER KEYBOARD and type in "R." A message appears that tells you to "press data out on reader now!"

w) Press the DATA OUT key on the Microwell reader. This will cause all of the data collected to be transferred to the computer.

x) Enter a sample number for each sample and press the ENTER key.

y) When the last sample number is entered, hit the ENTER key and the calculated ppm for each standard and sample will appear on the screen.

z) Record the results for each sample along with the correlation coefficient, slope, and y-intercept data on a data sheet.

NOTE: The correlation coefficient values must read .98 or higher to ensure accurate results. If the correlation value is less than .98, rerun the test. In addition, contact Neogen if the correlation coefficient is consistently below .98. Moreover, the slope value must read -2.0 (+ OR - 0.5). If the slope value consistently reads outside these tolerances, contact Neogen as soon as possible to report these findings. Do not certify results if the correlation coefficient is less than 0.98 or the slope value is out of tolerance.

3) Reporting and Certifying the Results.

a) Report all results on the pan ticket and inspection log to the tenth ppm. Refer to Section 15 (Certification) of this instruction for detailed certification procedures.

b) Results over the conformance limit are reported as estimates (e.g., 7 ppm-Estimated) unless a supplemental analysis is performed. (Refer to Section 14 (Supplemental Analysis) of this instruction for detailed procedures).

c. Equipment and Supplies.

(1) Test kit components

(a) monoclonal antibody-coated microwells

(b) red-marked mixing wells

(c) yellow-labeled bottle(s): DON control

(d) blue-labeled bottles: conjugate solution

(e) squeeze tubes: hydration solution

(f) green labeled bottle: substrate solution

(g) red-labeled bottle: stop solution

(2) Mixing Bags - 18-ounce Nasco Whirlpack bags; Fisher Scientific No. 01-812-6C, or similar type of sealable plastic bag

(3) Nalgene funnels - 80 mm top I.D., stem 30 mm, stem O.D. 18 mm; American Scientific Products No. F7465-2

(4) Plastic beakers - 250 mL plastic

(5) Cylinders - Polypropylene, graduated, 250 mL capacity

(6) Carboy - Nalgene, polyethylene, with spigot, 2 gallon capacity; Fisher Scientific No. 02-936-6A

(7) Filter paper - 24 cm diameter; Whatman No. 1, or equivalent

(8) Timer - 10 minute capacity

(9) Markers - Sharpie or equal (permanent ink that will not wash off)

(10) Absorbent material - Kim wipes or paper towels

(11) Wash Bottle 250 mL plastic squeeze bottle

(12) Microwell Strip Reader BioTek EL301 or equivalent

(13) IBM Compatible Computer

(14) Multichannel Pipettor - TiterTek 12 channel or equivalent

(15) Pipettor and Pipette Tips (100 μl) - Pipetteman, MLA or equivalent

(16) Pipettor and Pipette Tips (1mL) - Pipetteman, MLA or equivalent

(17) Microwell Holder

(18) Deionized or distilled water

(19) Strand Sizer or similar type shaking device

(20) Whirlpack Bag Rack; Fisher Scientific No. 01-812-5E, or equivalent

(21) Bond Elut SPE Cartridge, CN-E, 100 mg/1mL; Varian Sample Preparation Products No. 1210-2007 (For malted barley only)

(22) Vac Elut 10 with collector rack for 16 x 100 mm test tubes; Varian Sample Products No. 1223-4039 (For malted barley only)

d. Waste Disposal.

After the test has been completed, the remaining sample extract may be poured down the drain and solid material discarded in a trash can.

e. Storage Conditions.

Test kits should be refrigerated at temperatures between 36° F and 46° F.

12. ROMER DON FLUOROQUANT TEST METHOD

The extraction solution and other materials used in the Fluoroquant test kit necessitates the use of separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this directive to ensure a safe and efficient work environment.

a. Preparation of Extraction Solvent (84 Percent Acetonitrile Solution).

Make up the solution by using the ratio of 84 parts acetonitrile to 16 parts deionized/distilled water. Prepare the 84 percent acetonitrile solution by adding 840 mL acetonitrile to 160 mL of distilled or deionized water. Mix well. Label the solution bottle and keep it tightly capped when not in use. If the amount of solution being prepared needs to be adjusted based on the workload at individual locations, make sure that 84 parts acetonitrile to 16 parts deionized/distilled water ratio is maintained.

b. Extraction Procedures.

(1) Place 50 grams of ground sample into blender jar.

(2) Add 200 milliliters (mL) of acetonitrile/water (84/16) and blend on high for 3 minutes.

(2) Filter into a sample container using coffee filters or Whatman No. 1 filter paper.

c. Purification Procedures.

NOTE: All solution transfers may be carried out using adjustable automatic pipettors with disposable tips. Care should be taken to make sure that the tips used are large enough to hold the volume being transferred. Make sure that they are securely attached to the pipettor.

(1) Place 4 mL of extract in a 15 x 85 culture tube. Insert a MycoSep #225 column into the top of the culture tube and slowly (20 seconds) push to the bottom of the tube. (Note: Use 6 mL of extract and a MycoSep #227 column for malted barley samples. Take 30 seconds to push the extract through this column.)

(2) Transfer 1.5 mL of each purified sample extract to a 12 x 75 mm cuvette. Use a clean pipette tip for each transfer.

d. Calibrators and Control Preparation.

(1) Allow calibrator and control solutions to come to room temperature.

(2) Invert each calibrator standard bottle and control standard bottle three times to mix thoroughly.

(3) Transfer 1.5 mL of the green labeled calibrator solution to a clean 12 x 75 mm cuvette.

(4) Using a clean tip, transfer 1.5 mL of the red labeled calibrator solution to a clean 12 x 75 mm cuvette.

(5) Using a clean tip, transfer 1.5 mL of the control (yellow labeled) solution to a clean 12 x 75 mm cuvette.

(6) Cap the calibrator solutions tightly and store in the refrigerator.

(7) Proceed with the analysis, treating samples, calibrators, and control identically.

e. Evaporation Procedures.

Evaporate each sample, calibrator, and control to dryness using a vacuum manifold and dry bath set at 70°C. Note: To decrease the evaporation time, turn off the vacuum to the manifold for the rows that are not being used.

f. Derivatization Procedures.

(1) Add 1.5 mL of Reagent A to all sample tubes, calibrators, and control.

(2) Add 50 microliters (μl) of Reagent B to all sample tubes, calibrators, and control. Cap the tubes and mix contents with a vortex for 10 seconds.

(3) Heat the tubes in a 50°C bath for 10 minutes.

(4) Remove tubes from the bath and cool to room temperature. Read the samples in the fluorometer within 1 hour. Note: Cuvettes may be placed in tap water for 30 seconds to cool. Dry the outside wall of the cuvette completely before placing in the fluorometer.

g. Fluorometer Reading.

(1) Calibrate the fluorometer using the following procedure:

(a) Turn on the power (no warm-up is necessary).

(b) Change the date or time - If correct, press "CONTINUE" key.

(c) When asked for Test Delay Time, enter "2" and press the "ENTER" key.

(d) When asked for answer format, select "DECIMALS."

(e) When asked for measurement units, select "ppm."

(f) At the "insert red vial" prompt, place the appropriate calibrator cuvette into the sample well.

(g) When asked for the calibrator value, enter the appropriate value (refer to the card supplied with the calibrators for the red value) and press the "ENTER" key.

(h) When asked to "remove the red vial," remove the calibrator tube from the sample well.

(i) At the "insert green vial" prompt, place the appropriate calibrator cuvette into the sample well.

(j) When asked for the "blank value," enter the appropriate value (refer to the card supplied with the calibrators for the green value) and press the "ENTER" key.

(k) When asked to "remove the green vial," remove the calibrator tube from the sample well.

(l) At the "insert test vial" prompt, place the control cuvette into the sample well.

(m) The fluorometer will now display the value for the control vial.

(n) Compare the value of the control with the values listed on the card. If the control value is within the specified range, the fluorometer is ready to analyze samples. If the value is outside of the specified range, rerun the red, green, and yellow calibration cuvettes. If the control value still exceeds the specified range limit, contact Romer Labs.

(o) Press the "ENTER" key. The fluorometer is now ready to analyze samples.

h. Reading the Results.

To determine the DON concentration insert the cuvette containing the sample portion into the sample well of the fluorometer. The DON concentration will appear on the display after the appropriate 2-second delay. Read the results.

i. Reporting and Certifying the Results.

(1) Report all results on the pan ticket and inspection log to the tenth ppm. Refer to Section 15 (Certification) of this instruction for detailed certification procedures.

(2) Results over the conformance limit are reported as estimates (e.g., 7 ppm-Estimated) unless a supplemental analysis is performed. (Refer to Section 14 (Supplemental Analysis) of this instruction for detailed procedures.)

j. Equipment and Supplies.

(1) Test kit components

(a) glass culture tubes (15 x 85 mm) - 25 tubes per test kit

(b) MycoSep #225 (or #227 for malted barley) Columns

(c) glass cuvettes and caps (12 x 75 mm) - 50 per test kit

(d) Reagent A - Ethylenediamine in methanol

(e) Reagent B - Zirconyl Nitrate in methanol

(f) Calibrator solutions (red label, and green label) plus control solution (yellow label)

(2) Blender - Oster Mixer, Model 848-31A or Waring Blender with S.S. blender container or similar, the unit must be explosion proof

(3) Cutting Assembly - Process unit with sealing ring for Oster Mixer, Model 848-31A; Oster Corp. 937-45 or Eberbach blender jar or similar

(4) Bottom Cap - Threaded for Oster Mixer, Model 848-31A; Oster Corp. No. 937-46

(5) Square type jar - Designed to fit above

(6) Nalgene funnels - 80 mm top I.D., stem 30 mm, stem O.D. 18 mm; American Scientific Products No. F7465-2

(7) Plastic beakers - 250 mL plastic

(8) Cylinders - Polypropylene, graduated, 250 mL capacity

(9) Carboy - Nalgene, polyethylene, with spigot, 2 gallon capacity; Fisher Scientific No. 02-936-6A

(10) Extraction Solvent - Acetonitrile/water (84/16).

(11) Filter Paper - standard coffee filters or Whatman No. 1

(12) Fluorometer with printer - Romer RL 100 (# FQ 1002) or equivalent (Vicam Series III and IV)

(13) Vortex Mixer - Romer #EQP 8113

(14) Vacuum Pump and Trap - Romer # EQP 8106

(15) E-Vap Evaporator - Romer # EQP8188

(16) Test Tube Rack - Romer # EQP 8193

(17) Thermometer - Romer # EQP 8174

(18) Dry Bath with Heating Block - Romer # EQP 8180 / EQP 8181

(19) Pipette and tips - 50 μl

(20) Pipette and tips - 1.5 mL

k. Storage Conditions.

(1) MycoSep #225 and #227 columns - Room temperature in a drawer or box.

(2) Reagents A & B - shipped in amber bottle, cap tightly and store in a temperature controlled area (between 40° and 80° F). Do not freeze. Reagents should stay stable for 6 months.

(3) Calibration and control solutions - shipped in amber vials, cap tightly and store in the refrigerator. Solutions should stay stable for 6 months.

l. Waste Disposal.

(1) Transfer sample extract solutions (acetonitrile/water) and derivatization solutions into a liquid waste container for disposal. Follow SOP, established by the field office, for handling and disposing of hazardous waste.

(2) Transfer unextracted and filtered sample, used filter paper, cuvettes, caps, and #225 or #227 columns into the normal solid waste container for routine disposal.

13. ROMER ACCUTOX™ TEST METHOD

The extraction solution and other materials used in the AccuTox™ test kits do not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this directive to ensure a safe and efficient work environment.

a. Extraction Procedures.

(1) Place 50 grams of ground sample into a clean plastic or glass container.

(2) Add 250 milliliters (mL) of distilled or deionized water.

(3) Seal or cover the mixing container and shake (by hand or mechanically) for 3 minutes.

(4) Allow the sample residue to settle.

(5) Unseal or remove the cover from the container and pour the extract through filter paper (standard coffee filters or Whatman No.1) into a sample jar labeled with the sample identification.

b. Test Procedures.

(1) Allow reagents, antibody coated tubes, and sample extracts to reach room temperature prior to running test (approximately 1 hour).

(2) Place the appropriate number of labeled antibody-coated tubes into the gripper tube rack. Reseal the unused tubes in a zip-lock bag with desiccant.

(3) Pipette 0.5 mL of the zero calibrator, control, and samples directly into the bottom of the antibody coated tubes without touching the sidewalls. Completely discharge the pipette by depressing the plunger with the thumb to the second stop (all the way down).

(4) Pipette 0.5 mL of the enzyme conjugate into each tube. The conjugate must be pipetted (completely releasing the thumb after each addition) down the sides of the tubes. Start a timer set for 15 minutes as soon as conjugate has been added to the first tube.

(5) Shake the rack using a circular motion for approximately 5 seconds.

(6) At the completion of the 15-minute incubation period, dump the contents of the tubes into the appropriate waste container. Fill the tubes to overflowing and forcibly rinse with the wash solution. Completely empty the tubes after the rinse. Repeat this process three more times for a total of four washes. (It is very important not to underwash the tubes. Over washing will not affect the test.)

(7) Following the last wash, invert the tubes and forcibly tap onto absorbent paper several times to remove all of the wash solution. (It is important to remove as much wash as possible.)

(8) Pipette 0.5 mL of the substrate into each tube. The substrate must be pipetted (completely releasing the thumb after each addition) down the sides of the tubes. Start a timer set for 5 minutes as soon as the substrate has been added to the first tube. Swirl the rack in a circular motion for approximately 5 seconds to mix. Solutions should all turn blue after substrate has been added.

(9) Add 0.5 mL of stop solution to each tube by pipetting down the sides of the tubes. Swirl the rack in a circular motion for approximately 5 seconds to mix. All solutions should turn yellow after adding the stop solution.

(10) Make sure that the spectrophotometer is set at 450 nm. Run a blank with a clean unscratched test tube filled with fresh distilled or deionized water.

(11) Zero the spectrophotometer prior to reading the tubes. Make sure that there are no air bubbles in the blank tubes before zeroing.

(12) Wipe each tube with a lint free towel before reading and allow a few seconds for the specrophotometer reading to stabilize before printing the absorbance level reading.

(13) Read and record absorbance levels of the calibrator, control, and samples.

(14) Calculate results using log/logit data computer program with factory calibration included with kit.

c. Spectrophotometer Calibration Procedure.

(1) Turn on the spectrophotometer by using the ON/OFF key. After turning on the power, the spectrophotometer goes through a self test. **Note: The lid must be closed.**

(2) Using the dial on the right side of the spectrophotometer, dial in 450 nm.

(3) The spec will then display “Enter Program #.” At this time press 0 and the “Enter’ button.

(4) P O will then be displayed. Take the blank tube filled half way with distilled or deionized water and place into the well. Cover the tube with the small cylinder cover supplied.

(5) Press the key labeled “ Zero.”

(6) The spectrophotometer is now ready for calibrators and samples.

(7) After placing calibrator or sample into the well and covering, wait a few seconds (1-5 seconds) for the reading to stabilize.

(8) Turn on the printer and verify that the printer on line light is lit. Press the “Shift” key, then the “Print” key (blue writing) to print the absorbance level readings.

d. Reporting and Certifying the Results.

(1) Report all results on the pan ticket and inspection log to the tenth ppm. Refer to Section 15 (Certification) of this instruction for detailed certification procedures.

(2) Results over the conformance limit are reported as estimates (e.g., 7 ppm-Estimated) unless a supplemental analysis is performed. (Refer to Section 14 (Supplemental Analysis) of this instruction for detailed procedures.)

e. Equipment and Supplies.

(1) Test kit components

(a) Antibody coated tubes

(b) Conjugate

(c) Substrate

(d) Zero calibrator standard

(e) Control solution

(f) Stop solution

(g) Wash solution

(2) Hach Spectrophotometer

(3) 500 μl pipettor with tips

(4) Gripper test tube rack

(5) Timer - 15 minute capacity

(6) Plastic squirt bottle for wash solution

(7) Sealable plastic bags or plastic/glass containers with tight fitting lids

(8) Distilled or deionized water

(9) 100 mL graduated cylinder

(10) Filter paper (standard coffee filters or Whatman No.1)

f. Waste Disposal.

After the test has been completed, the remaining sample extract may be poured down the drain and solid material discarded in the trash can.

g. Storage Conditions.

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

14. SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as estimates (e.g., 7 ppm-Estimated). If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** (see the General Information section for specific limits for each test method) is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis gave an estimated value of 9.0 ppm and the conformance limit value was 5 ppm, in order to obtain a true value, dilute 5 mL of the original extract with 10 mL of the extraction solution (distilled/deionized water for the Neogen methods and the Romer AccuTox™ method, or acetonitrile/water for the Romer DON Fluoroquant method). The total volume is 15 mL. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the controls provided with the kits.

15. CERTIFICATION

a. General. Wheat, barley, corn, and oats are tested for DON under the authority of the United States Grain Standards Act (USGSA). Under the USGSA, DON results are recorded on the pan ticket, worksheet, or shiploading log and in the remarks section of the certificate.

The type of service requested and the test method used determine how DON results are recorded and certified.

Record the results of a **qualitative service** as being equal to or less than a threshold (e.g., 2 ppm) or as exceeding the threshold.

If a **quantitative method** is used to provide qualitative service, record the quantitative test results on the work records **to the tenth ppm** even though the results are certified as meeting or exceeding a threshold.

When quantitative test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as **"less than or equal to 0.5 ppm."** Test results **between 0.6 ppm** and the **conformance limit** are certified to the nearest whole ppm. **Upon request** of the applicant, results may be certified to the **nearest tenth ppm.**

Sections 800.125 and 800.135 of the regulations under the USGSA permit a review inspection on either official grade/factors or official criteria. When requested, a review inspection for official grade or official factors and official criteria may be handled separately, even though both sets of results are reported on the same certificate. When official grade or official factors and official criteria are reported on the same certificate, the review inspection certificate shall show a statement indicating that the review results are for official grade, official factors, or official criteria, and that all other results are those of the original, reinspection, or appeal inspection results, whichever is applicable.

Certify DON test results on grain in accordance with sections 800.160 through 800.166 of the regulations under the USGSA.

Upon the request of the applicant, separate certificates may be issued for grade and for DON when both are determined on the same lot.

b. Unit Trains. Samples may be obtained and tested on either an individual carrier basis or a sublot basis (up to five carriers per sublot). When articulated railcars are used, each car is tested as a sublot.

If an applicant requests DON testing on a sublot basis and the inspection for grade on the basis of individual carriers, factor only certificates are issued for the DON testing and separate grade certificates are issued for each carrier.

The applicant may request a review inspection when a material portion occurs due to DON. Refer to the Review Inspections section of this directive for guidelines for providing review inspection services. Review inspection results replace previous results when determining if a material portion exists.

(1) Individual Test Results. Unless otherwise specified by the applicant, certify each test result on a separate certificate. A certificate may represent a single carrier or a sublot from up to five carriers. The sublot certificate lists the identities of the carriers comprising the sublot.

(2) Combined Test Results. At the request of the applicant, individual test results that do not exceed the maximum acceptable DON limit (e.g., 2 ppm) may be combined in the case of qualitative results or averaged in the case of quantitative tests and reported on a single certificate. Results exceeding the maximum DON limit are certified separately for each test result. Issue a certificate for the averaged results and issue a separate certificate for any sublot exceeding the maximum DON limit.

c. Export Shiplots. Record individual sublot results on the inspection log to the nearest tenth ppm for quantitative analysis. If requested by the applicant, quantitative results used as a screening process are reported on the inspection log as screening results (exceeding or less than or equal to the maximum level) provided that laboratory records are maintained in actual ppm.

A material portion occurs when the sublot result exceeds the limit specified by the load order. The applicant may request a review inspection when a material portion occurs due to DON results. Refer to the Review Inspections section of this directive for guidelines for providing review inspection services. Review inspection results replace previous results when determining if a material portion exists.

If the review inspection process does not remove a material portion designation due to DON, the applicant may:

(1) leave the material portion on the vessel and receive a separate certificate;

(2) return the grain from a shipping bin to the elevator; and/or

(3) discharge the material portion along with additional grain in common stowage equivalent to half the material portion quantity.

When sublot samples are tested using a **qualitative method**, certify the shiplot results as equal to or less than the maximum limit (e.g., 2 ppm). Include on the same certificate the composite sample results (qualitative or quantitative) if a composite sample was also tested. If some sublots were reviewed using a quantitative method, continue to certify the shiplot as equal to or less than the maximum limit.

When sublot samples are tested using a **quantitative method**, certify the shiplot based on the mathematical or weighted average of the accepted sublot results (see Book III of the Grain Inspection Handbook, section 2.8, "Determining Mathematical or Weighted Average"). In addition, include on the same certificate the composite sample results (qualitative or quantitative) if requested. Certify material portions separately.

d. Approved Statements. Upon request of the applicant, the term vomitoxin may be used in lieu of the term DON in the certification statements.

(1) Qualitative Service.

For qualitative service, certify results as being equal to or less than a threshold (e.g., 2 ppm) or as exceeding the threshold.

"DON exceeds 2 ppm."

"DON equal to or less than 2 ppm."

(2) Quantitative Service.

Use one of the following statements for certifying DON on a quantitative basis.

(a) When DON results are less than or equal to 0.5 ppm:

"DON less than or equal to 0.5 ppm."

(b) Certify results between 0.6 ppm and the conformance limit to the nearest whole number in ppm.

"DON (result rounded to the nearest whole number) ppm."

(c) Results over the conformance limit are reported as estimates unless a supplemental analysis is performed.

"DON 6 ppm-Estimated."

NOTE: Do not show "estimated" if solution was diluted and supplemental analysis performed.

(d) Board Appeals performed by the High Pressure Liquid Chromatography (HPLC) method are certified to the nearest tenth ppm.

"DON (record actual results to the nearest tenth) ppm. Results based on High Pressure Liquid Chromatography Method."

e. Other Statements.

(1) Use this statement when the applicant requests the type of test shown on the certificate:

"Results based on (indicate type of test used) method."

(2) When certifying multiple DON results on the same certificate and the results are based on different sample types the certificate must reflect the difference. As a guideline, the multiple results are shown as follows:

"Sublot sample results: DON equal to or less than (threshold) ppm."

"Composite sample result: DON (actual result) ppm."

(3) At the request of the applicant, use the following statement when DON is not detected using a quantitative method (0.0 ppm).

"DON not detected."

If sublot results are combined and averaged and the lot average is equal to 0.0 ppm, but an individual sublot result exceeds 0.0 ppm, then the statement "DON less than or equal to 0.5 ppm" must be used.

(4) At the request of the applicant, certify quantitative results between 0.6 ppm and the conformance limit to the tenth ppm.

"DON (result in tenths) ppm."

(5) Upon request of the applicant, one of the following statements may precede the applicable results statement when test results are equal to or less than the specified threshold.

"The DON result is negative." OR "Negative DON."

(6) Upon request of the applicant, convert and certify the ppm result to parts per billion (ppb) using an approved statement. To convert ppm to ppb, multiply the ppm result by 1000.

"(Actual ppm result) ppm is equivalent to (converted ppb results) ppb."

(7) Upon request of the applicant, convert and certify results in milligrams per kilogram (mg/Kg) or micrograms per kilogram (μg/Kg). Use the following equivalents to determine mg/Kg or μg/Kg:

ppm = mg/Kg ppb = μg/Kg

NOTE: These certification statements may be modified as deemed necessary.

f. Reinspection, Appeal, Board Appeal Certificates.

(1) Results are reported on the same kind of certificate issued for the original service and supersede the previously issued inspection certificate.

Enter the following statement on the reinspection/appeal/board appeal certificate:

"This certificate supersedes Certificate No. (number) dated (date)."

(2) The superseded certificate is null and void as of the date of the subsequent (reinspection/appeal/board appeal) certificate.

"The superseded certificate has not been surrendered."

(3) When a file sample is used, enter the following statement on the reinspection/appeal/board appeal certificate:

"Results based on file sample."

(4) When reporting more than one official result on the same certificate but at different levels of inspection, explain this condition using one of the following applicable statements:

"(Grade, factor, or official criteria) results based on (new/file) sample. All other results are those of the original inspection service."

"(Grade, factor, or official criteria) results based on the appeal inspection. All other results are those of the (original inspection/reinspection) service."

"(Grade, factor, or official criteria) results based on the Board appeal inspection. All other results are those of the (original inspection/ reinspection/appeal inspection) service."

16. QUALITY ASSURANCE PROGRAM

The Technical Services Division (TSD), located at the Kansas City Technical Center, conducts a DON check sample program for all specified service points and laboratories providing testing services. TSD is responsible for preparing and distributing check samples each quarter to all official DON testing locations, analyzing check sample results, notifying field locations of any results indicating problems, and releasing a quarterly summary report to all participating laboratories. Field offices are responsible for routine supervision to assure all laboratories in their circuit provide accurate results. The TSD check sample program is designed to test the capability of the official system and to monitor the accuracy of approved testing methods. The check sample program provides limited performance information that can be used to supplement the routine supervision of official personnel performing testing services.

/s/ David Orr

David Orr, Director
Field Management Division